# Effect on lung permeation of various formulation efforts invested in the inhaled antibiotic apramycin as measured in the *ex vivo* isolated, perfused, and ventilated rat lung

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## Summary

To increase the effect duration and local efficiency of the inhaled antibiotic apramycin lung permeation of three different formulations was measured in the isolated, ventilated, and perfused lung of the rat (IPL). The studied formulations of apramycin were; one nebulized reference solution (Ref), one dextran-based nanoparticle suspension (KuDa), and one micronized powder formulation coated with phospholipid (DSPC). All three formulations generated aerosols in the PreciseInhale® exposure platform with mass-median aerodynamic diameters (MMAD) ranging from 2.8 to 3.6 µm. Lungs were exposed to nebulized- or dry-power aerosols for up to 7 min. Fractions of single-pass perfusate were collected for 120 min after which BAL fluid, lungs, and perfusate were collected for quantitation of apramycin. Results show that whereas the nebuliser reference solution had a distinctive Cmax during the first hour after dosing, both engineered formulations of apramycin had a slower and nearly constant permeability from 10 to 120 min after the initial break-through to the perfusate. At 120 min the overall rate of permeation was similar for Ref and DSPC formulations with a 4 hr half time, whereas KuDa had a slower 6 h half time. In summary, it is possible to increase the lung retention of water soluble apramycin by either crystal coating or dextran entrapment of the API.

## Key Message

It is possible to increase lung residence time of the water soluble antibiotic apramycin by incorporating the substance into a surfactant-coated powder or a dextran-based nanosuspension. Both engineered formulations removed the distinctive Cmax of the reference solution aerosol, and the dextran-based formulation also reduced over all permeation rate by 30%.

## Introduction

This work was conducted within the APRINHA project [1], the goal of which was to create up to five different formulations of the antibiotic apramycin [2] (solubility in water; >200 mg/mL, permeability; $1.6x10^{-7}$ cm/s Calu-3 ALI apical to basal) to achieve a longer residence time in the lungs of infected patients. In the current paper the results of three different apramycin formulations are reported: 1) Reference apramycin formulation (Ref); 2) Dextran-based single-chain polymer nanoparticles (KuDa); 3) Micronized apramycin with a surface-active coating (DSPC). One prime instrument for selection of best formulation is the lung pharmacokinetics (PK) of apramycin following inhalation into the *ex vivo* tracheally intubated model of the isolated perfused and ventilated lung of the rat (IPL). This presentation shows lung specific PK data of apramycin following inhalation administration of formulations 1-3 to *ex vivo* lungs from healthy rats.

## Experimental methods

The following materials were used: 1) Reference apramycin formulation: a liquid formulation for nebulization, containing 75 mg/mL apramycin dissolved in purified water and pH adjusted with sulfuric acid to pH 5.6; Provided by RISE, Department of Chemical Processes and Drug Development, Forskargatan 18, SE-151 36 Södertälje, Sweden; 2) KuDa-Apry: lyophilized powder (400 mg per one vial) containing 30 wt% apramycin; Provided by CIDETEC Nanomedicine, Parque Cientifico y Tecnológico de Gipuzkoa P$^{O}$ Miramón, 196 20014 Donostia-San Sebastián (Gipuzkoa), Spain; The

content of each vial was reconstituted with aq. pro inj. to obtain a liquid formulation for nebulization, in which the concentration of the KuDa/apramycin formulation- and apramycin only were respectively, 100 mg/mL and 30 mg/mL; 3) DSPC-coated apramycin: micronized dry powder containing 96 wt% apramycin; Provided by Research Institute of Sweden (RISE), Department of Chemical Processes and Drug Development. For the *ex vivo* IPL exposures to study formulations 1 and 2 (solutions), aerosols were generated using the nebulizer generator (Figure 1A) of the PreciseInhale® exposure platform (Inhalation Sciences Sweden AB, Stockholm, Sweden). Aerosol was generated using the downward acting Aeroneb Pro nebulizer (Aerogen, Galway, Ireland); mesh size of approx. 4 µm and 10 µm was used for respectively, formulation 1 and 2.The nebulizer output was reduced to the range of 1-10% of continuous operation (corresponding to a feed liquid consumption of about 3-30 µL/min), to better match exposure requirements of the ventilating rat lung. The nebulizer was placed at the top of an aerosol holding chamber connected to an exposure block with a three-way junction exposing the rat lungs in a flow-past configuration. The aerosol was delivered to the exposure module as one spray pulse per second into a constant carrier airflow regulated by a downstream, precision-controlled vacuum source. A dried aerosol was obtained by shrinking the droplets towards their dry residual size, using room air dehumidified by passage of a column with silica gel [3]. For the exposures to study formulation 3 (powder), aerosols were generated with the powder aerosol generator (Figure 1B) of the PreciseInhale® using pressurized air [4] . Particle size distributions of generated nebulizer- and powder aerosol were determined using a Marple 8-stage cascade impactor (MSP Corp., Shoreview, MN, USA) at a flow rate of 2 L/min, and mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were derived [5]. The IPL experiments (performed at Inhalation Sciences) were approved by the local ethics review board (Linköpings djurförsöksetiska nämnd Dnr 11479-2020). Three groups of male CD IGS (Sprague Dawley) rats (n=3 per group; Charles River Laboratories, Italy) with the body weights 338 ± 12 g, 345 ± 31 g and 360 ± 15 g (mean ± Standard Deviation) were used for the experiments with respectively, formulation 1, 2 and 3.

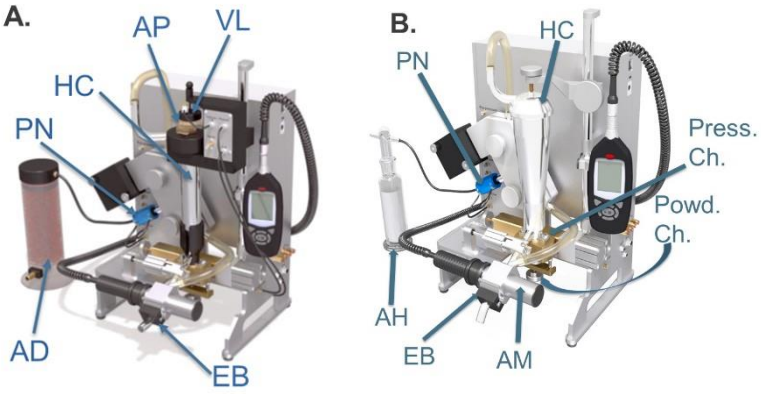


**Figure 1- The nebulizer- (A) and powder- (B) aerosol generator set-up of PreciseInhale; (A): AP, Aeroneb Pro mesh nebulizer; VL, Airtight lid with a slight vacuum; AD, Air dryer; (B) Press.Ch., Pressure chamber; Powd.Ch., Powder chamber; AH, Air humidifier; (A-B): HC, Aerosol holding chamber; PN, Pneumotach; AM, Aerosol monitor; EB, Exposure block (connection to exp. target)**

The IPL (Figure 2) was prepared as described elsewhere [6, 7]; Briefly, the negative pressure ventilation and the single-pass perfusion through the pulmonary artery at a constant hydrostatic pressure was used. For each formulation, the targeted dose of apramycin deposited per lung was 200 µg. After dosing the lungs were maintained for a 120-min period, during which perfusate was collected with a sample collector at the following time points: 1.5, 3, 4, 5, 6, 8, 10, 12, 15, 30, 45, 60, 90, 120 minutes from the start of aerosol exposures (t=0). Directly afterwards the lungs were flushed twice with saline, 1 mL each time. After the BAL procedure, whole lungs were collected for quantification of the amount of apramycin retained in lung tissues at the end of the perfusion period.

**Figure 2- A prepared isolated, perfused and ventilated rat lung (IPL); Before aerosol exposures, the Hans Rudolph pneumotachograph was removed and the tracheal catheter of the IPL was connected to the PreciseInhale at the exposure block (Figure 1, EB).**

The LC-MS/MS quantification of apramycin in lung perfusate-, BAL- and tissue samples was performed by RISE, Sweden. PK analysis was performed as previously reported [8].

**Results and discussion**

Highly dispersed aerosols were obtained from all three study formulations when aerosolized with either the PreciseInhale® nebulizer- or powder aerosol generator (Table 1).

**Table 1- Mass Median Aerodynamic Diameter (MMAD), Geometric Standard Deviation (GSD) and theoretical deposition fractions (MPPD v. 3.04) of the study aerosols**

| Formulation | MMAD (µm), mean | GSD, mean | Fdep[1], mean | | | Number of exp. |
|---|---|---|---|---|---|---|
| | | | Total [2] | Bronchial [4] | Alveolar [5] | |
| 1) Reference apr. form. (solution) | 2.84 | 1.87 | 0.42 | 41% | 59% | 3 |
| 2) KuDa-Apry (suspension) | 3.61 | 2.16 | 0.49 | 51% | 49% | 3 |
| 3) DSPC-coated apr. (powder) | 2.99 | 2.21 | 0.43 | 46% | 54% | 3 |

[1]The theoretical fractional deposition of a study aerosol when inhaled by the rat IPL with the following ventilation pattern: endotracheal breathing mode, TV 1.5 mL, 75 breaths/ min.

[2] The total fractional deposition of aerosol.

[3] The normalized fractional deposition of aerosol in the conducting airways.

[4] The normalized fractional deposition of aerosol in the alveolar region.

The achieved total deposition of apramycin per rat lung tested for formulations 1, 2 and 3 were respectively; 156 ± 14 µg, 151 ± 22 µg and 117 ± 13 µg (mean ± Std Dev, for n=3 per group).

There was a distinctive difference in permeation of apramycin between the study formulations (Figure 3 and Figure 4). Whereas fully dissolved apramycin in aqueous solution permeated with an elevated Cmax peak during the first hour; for the two engineered formulations this peak was suppressed and replaced with a constant rate of permeation for the 2 h sampling period (Figure 3).

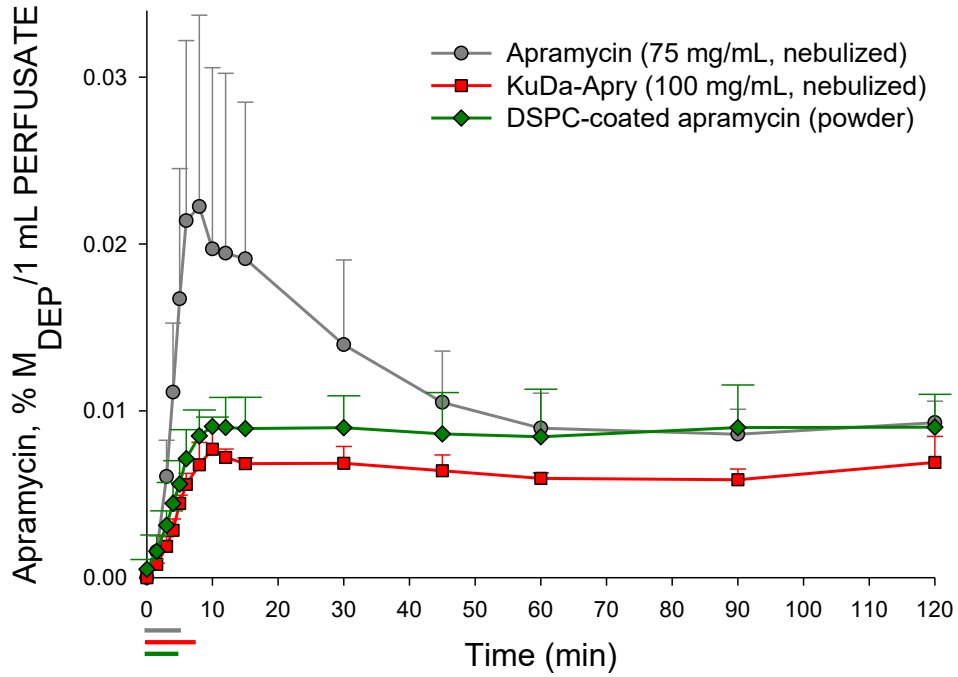


**Figure 3 - Apramycin: Concentration (normalized to the initially deposited dose according to LC-MS/MS) in the single-pass lung perfusate vs. time; The grey bar: exposure duration = 5 min for Reference apramycin formulation; The red bar: exposure duration = 7 min for KuDa-Apry; The green bar: exposure duration = 4.2 (±0.2) min (mean ± Std Dev; n=3) for DSPC-coated apramycin**.

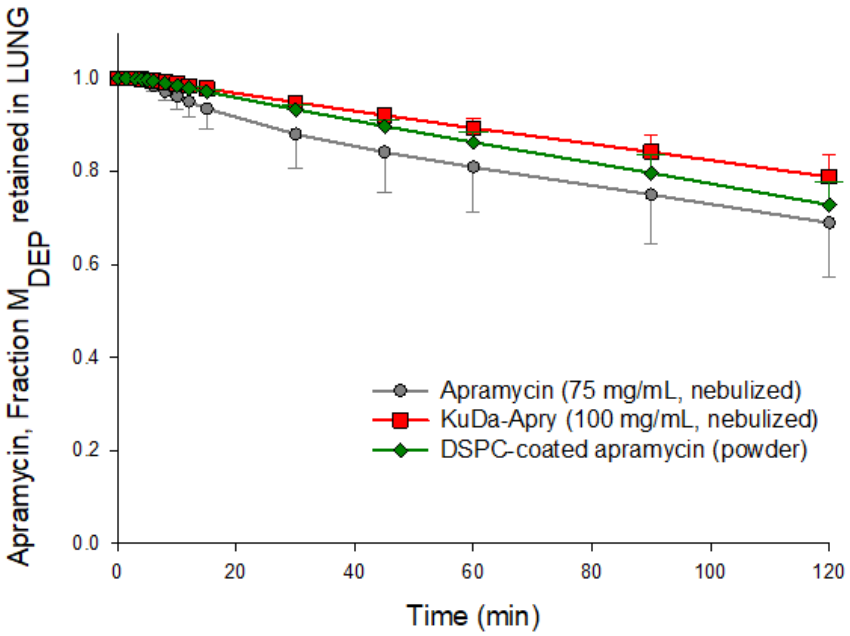


**Figure 4 - Apramycin: Retention (normalized to the initially deposited dose according to LC-MS/MS) in the lungs vs. time**

## Conclusion

Compared to a reference solution of nebulized apramycin (API), permeation of this water-soluble antibiotic in the lungs can be decreased by incorporating the API into various engineered formulations. Permeation can be decreased both by removing a prominent Cmax peak and by slowing the overall rate of permeation to the circulation. Both engineered formations were able to completely suppress a protruding Cmax peak, whereas only the dextran-based formulation was able to reduce the overall rate of permeation throughout the 2 h sampling period of perfusate. This data set provides important information on the behaviour in the lungs of inhaled substances belonging to the Class III of highly soluble, slowly permeating inhalants of the newly proposed iBCS classification system for inhaled pharmaceuticals [9]. While the classification system provides an excellent structure for the general behaviour of inhalants in the lung, the current data hints at a greater complexity in the resulting pharmacokinetics, in particular for engineered formulations that likely affects dissolution/desorption rates but not the solubility in the lung lining layer.

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